ISOTONIC AND ISOMETRIC THERMAL CONTRACTION OF HUMAN DERMIS. III. SCLERODERMA AND CICATRIZING LESIONS*†

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The pathogenesis of scleroderma has been attributed to alterations within the collagen because clinically there is sclerosis of the tissue and histologically the principal findings are observed as changes in the size and staining of collagen bundles. Recent evidence, however, obtained from amino-acid determinations, roentgen-ray diffraction patterns, histochemical technics, and electron microscopy, suggests that the collagen may be involved only secondarily or possibly not even at all. (1, 2) Relatively little is known about the physical and structural characteristics of sclerodermatous collagen, and considerable confusion still exists regarding the age of the collagen in the involved tissue.

This study was undertaken to investigate the structural stability of the collagen in scleroderma by a study of its hydrothermal shrinkage properties. In addition, since we have been able to characterize the age of collagen by its shrinkage qualities, (3) the age of the collagen in scleroderma was compared to that of collagen from normal individuals, and to that of collagen from cicatrizing lesions.

MATERIAL AND METHODS

Samples of skin were taken from 15 patients with localized or generalized scleroderma (table I) and from nine patients with either scars or keloids (table II). The 15 cases of scleroderma included nine cases of acrosclerosis, four cases of localized morphea, and two cases of linear scleroderma, a variety of localized scleroderma. All but one of the patients who had acrosclerosis had a history of Raynaud's phenomenon with sclerosis of the skin beginning peripherally, and all but two had esophageal motility studies showing sclerodermatous change. None of the patients with morphea or linear scleroderma had any sign of systemic involvement, although one (case 1), in addition to linear scleroderma, had some joint and muscle pain.

The tissue samples were obtained from living patients, except that in one case of acrosclerosis the specimen was removed at the time of necropsy. The samples were removed so that the long axes of the specimens were parallel to the skin creases, the epidermis and adipose tissue were mechanically removed, and the samples were cut into uniform strips measuring 2 by 15 mm. Two of these strips, suspended side by side in a water bath, were used for experiment. One was attached to the arm of a kymograph and the other to a strain gauge. A previously described shrinkage technic was used. (4) By this technic, the shrinkage temperature (Ts), the amount of contraction, and the tension (both total and in relation to the temperature) could be measured and recorded simultaneously as the temperature of the water bath was slowly raised from 50° to 90° C.

RESULTS

Shrinkage Temperature.—The Ts from the 15 samples of sclerodermatous tissue varied from 61° to 63° C., with a slight increase with age (fig. 1). The values from all 15 patients fit well into the curve for normal individuals. No difference was found between the lesions of morphea and acrosclerosis, and no correlation existed between the duration of lesions and the amount of sclerosis.

Of the nine cicatrizing lesions studied, seven had shrinkage temperatures which were comparable to those of specimens of normal tissue from patients of the same age (fig. 1). The other two, one keloid and one scar, had shrinkage temperatures which were lower (58.0° and 61.0° C.) than would be expected of normal tissue from an individual of a similar age. Both of the lesions from which these samples were obtained had been present for only a short time, 3 and 5 months, respectively; whereas all of the other keloids and scars had been present for at least 1 year. In cases 4, 6, and 7 (table III) the Ts of the lesions was compared with that of the patients' own normal tissue. Of these, one patient (case 4) had a keloid, present for

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TABLE I

Hydrothermal Shrinkage Properties of Tissue From 15 Patients With Scleroderma

Case	Age, yr.	Sex	Diagnosis	Duration of sclerosis	Contraction (cm)*	Shrinkage temp. (° C).	Tension (gm)	
							Total	Per mm dermal thickness
1	10	F	Linear scleroderma	2 years	—	61.0	170	85
2	21	M	Acrosclerosis	8 months	8.0	62.5	220	88
3	26	F	Morphea	8 years	—	61.0	175	80
4	33	F	Morphea	1 month	—	61.5	195	97
5	39	M	Acrosclerosis	3 months	7.1	61.5	175	67
6	40	F	Acrosclerosis	2 years	—	62.5	140	76
7	41	F	Acrosclerosis	4 years	—	62.0	145	83
8	45	F	Acrosclerosis	3 years	5.5	61.5	215	102
9	48	F	Acrosclerosis	1 year	7.6	62.0	152	71
10	51	F	Acrosclerosis	3 years	8.6	62.5	260	118
11	53	F	Linear scleroderma	2 years	6.8	61.5	320	100
12	54	M	Acrosclerosis	3 months	7.8	62.5	300	105
13	54	F	Morphea	5 months	—	62.0	250	90
14	62	F	Morphea	3 months	5.0	63.0	265	106
15	62	F	Acrosclerosis	1 year	4.4	62.0	147	74

* Values, obtained by measuring distances on kymograph, are 9.5 times the true values.

TABLE II

Hydrothermal shrinkage properties of scars or keloids from nine patients

Case	Age, yr.	Sex	Diagnosis	Duration of lesion	Contraction (cm)*	Shrinkage temp. (° C.)	Tension (gm)	
							Total	Per mm dermal thickness
1	13	F	Scar	3 years	6.7	61.0	110	34
2	14	M	Keloid	8 years	8.1	60.0	170	53
3	17	M	Scar	2 years	7.1	60.5	102	30
4	20	M	Keloid	3 months	6.5	58.0	82	20
5	21	M	Keloid	2 years	9.2	62.0	137	40
6	26	M	Scar	2 years	9.0	61.0	105	38
7	28	F	Scar	1 year	8.0	61.0	100	37
8	48	M	Scar	5 years	9.0	62.5	165	60
9	78	F	Scar	5 months	8.6	61.0	130	52

* Magnified values—see note to table I.

3 months, which shrank at a temperature 2.5° lower than did normal tissue; another (case 7) had a scar, present for 1 year, which shrank at a temperature 1.0° lower than did normal tissue; and the third (case 6), had a scar, present for 2 years, which shrank at the same temperature as did normal tissue.

Amount of Contraction.—The contraction of sclerodermatous collagen, magnified 9½ times by the kymograph arm, varied from 4.4 cm (31 per cent of the magnified initial length of the specimen) to 8.6 cm (61 per cent of magnified initial length) (fig. 2). All 15 samples contracted less than the median values for normal collagen and in two cases of acrosclerosis and one of morphea, the contraction was below the range of values for the normal tissue. In general, the specimens with the greatest amount of sclerosis contracted the least, whereas those with the least sclerosis most nearly approximated the normal tissue.

The cicatrizing lesions were found to be within

the range of the normal controls, 6.5 cm to 9.2 cm (46 to 65 per cent of the magnified initial length), although specimens from seven of the nine cases gave average values which were 0.5 to 1.0 cm less than the normal median (fig. 2). In the three cases in which scar tissue and normal tissue were compared (table III), the amount of contraction of scar tissue in cases 4 and 6 was 2.9 cm and 1.8 cm less than that for normal tissue and in case 7 it was 1.0 cm more.

The curve produced on the graph by the shrinking collagen was the same for cicatrizing and normal tissue at all ages. In the sclerodermatous tissue with grade 3 and 4 sclerosis, the initial rise, immediately after the onset of shrinkage, was a little slower than that seen in normal tissue. Other than this the curves were identical.

Tension.—The rate of the tension development for sclerodermatous samples (fig. 3) was identical to that for normal adult tissue (fig. 4)—the tension, in both instances, continuing to rise at the 90° C. cut-off point. Likewise, the maximal tension values were distributed fairly evenly throughout and on both sides of the maximal tension curve for normal tissue (fig. 5). When the tension was corrected for dermal thickness (fig. 6), all but one of the scleroderma values lay within or above the range of values for normal individuals. The only value which fell below the normal values was obtained with a specimen from a 62-year-old woman (case 15), who had grade 4 acrosclerosis of one year's duration. No difference in either the rate of development or the amount of tension was found in specimens of morphea and

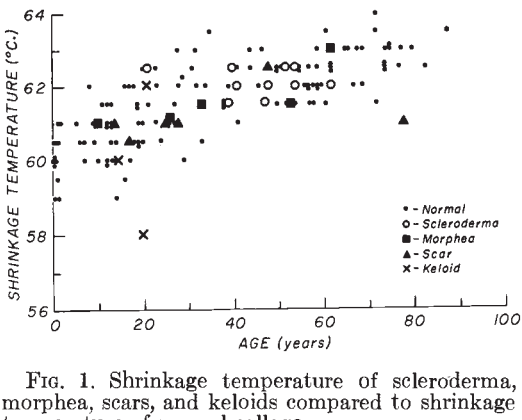

FIG. 1. Shrinkage temperature of scleroderma, morphea, scars, and keloids compared to shrinkage temperature of normal collagen.

TABLE III

Hydrothermal shrinkage properties of scars and keloids compared to those of normal tissue from same patient

Case*	Tissue	Contraction (cm)†	Shrinkage temp. (°C.)	Tension (gm)	
				Total	Per mm dermal thickness
4	Normal	9.4	60.5	155	62
	Keloid (3 mo.)	6.5	58.0	82	20
6	Normal	10.8	61.0	170	58
	Scar (2 yr.)	9.0	61.0	105	38
7	Normal	7.0	62.0	190	64
	Scar (1 yr.)	8.0	61.0	100	37

* Case numbers correspond with those of table II.

† Magnified values—see note to table I.

acrosclerosis, and there was no relationship to duration of sclerosis or to therapy. Samples from patients with the most severe sclerosis, in general, developed the most tension.

For the cicatrizing lesions, the rate of tension development was very similar to that found in normal young collagen; all but two of the samples reached a maximum tension before 90° C. (fig. 3). The two samples which did not reach a plateau early were the two oldest lesions, having been present for 5 and 8 years, respectively. In general, samples from lesions of the shortest duration reached the maximum tension earliest with some similarity existing when the age of the scar or keloid was compared with the samples from normal individuals of the same age (fig. 4). Maximal tension and tension per unit dermal thickness produced by the cicatrizing lesions were well below corresponding tension values for normal tissue from individuals of the same age (fig. 5 and 6). The only exception to this was the one keloid present for 8 years, which acted much like normal tissue from patients of the same age. In the three cases where the cicatrizing lesions were compared with normal skin from the same persons (table III), the tension of the lesion in each case was well below that of the normal tissue, the difference being greatest in a keloid of only 3 months' duration.

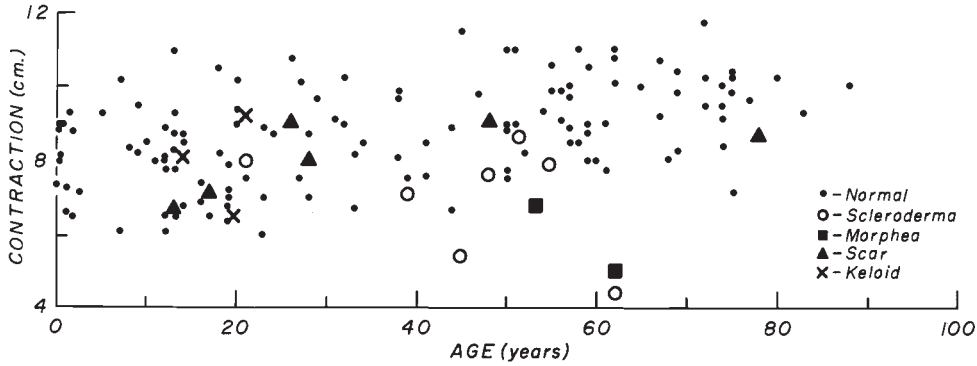

FIG. 2. Contraction of scleroderma, morphea, scars, and keloids compared to contraction of normal collagen.

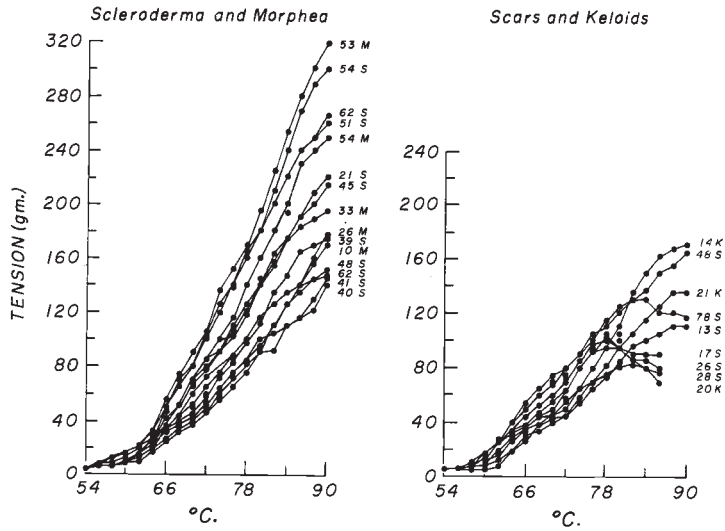

FIG. 3. Rate of tension development with temperature change in scars, keloids, and sclerodermatous collagen. Numbers to right of each chart refer to ages of patients in years. Initials indicate diagnoses: In left-hand chart, "M" means morphea and "S," scleroderma; in right-hand chart "K" means keloid and "S," scar.

COMMENT

Little difficulty arises either clinically or histologically in differentiating scleroderma from cicatrizing lesions, and yet several authors have stated that an increased production of young collagen accounts for the picture seen in both entities. Musso (5) in a study of early morphea found a definite increase in the amount of ground substance with increased numbers of new thin collagen fibrils interspersed throughout. Fibroblasts were either normal or decreased in number, and the ground substance was easily removed by trypsin. Because of these findings, he theorized that the interfibrillar ground substance, under altered physiochemical conditions, forms a stratum which can induce the occasional fibroblast to form increased amounts of young collagen and also can lead to the spontaneous deposition of new collagen in a manner similar to the well known reprecipitation experiments. (6–8) Keech, (9) in a study of collagen from patients with morphea and systemic scleroderma, also found a striking increase in the amount of mucopolysaccharides and of thin collagen fibers—much as one would expect to find in the dermis of infants or of very young children. He was unable, however, to find any significant difference in the col-

lagenase digestion of normal adult and pathologic tissue.

Others have not been so impressed with increased amounts of young collagen in scleroderma tissue. Fisher and Rodnan (2) found that sclerodermatous collagen, when examined by the electron microscope and studied enzymatically, was similar to collagen from normal controls of a comparable age. The collagen in healing wounds, on the other hand, was found to differ both morphologically and enzymatically from that of the normal and sclerodermatous tissue. He therefore concluded that the alterations in dermal collagen observed in scleroderma were the result of quantitative rather than qualitative changes in the homogeneity of the collagen and that they simply represented increased numbers of the normal collagen fibers. Stringer and Highton (10) found the shrinkage temperature in scleroderma to be the same as that of normal adult collagen whereas a keloid was found to have a shrinkage temperature slightly lower than that of normal adult tissue.

In the present study, there was a clear differentiation between the age of collagen in scars and that in sclerodermatous lesions. The collagen from scars and keloids, as would be expected, acted like young tissue which once formed proceeds to age the same as any other newly synthesized collagen. In those scars of only a few months' duration the collagen behaved much like that seen in infancy. After aging, the collagen assumed the shrinkage qualities of older tissue. A considerable difference exists for 8 or more years between the scar-tissue and the normal-skin collagen, and from the shape of the

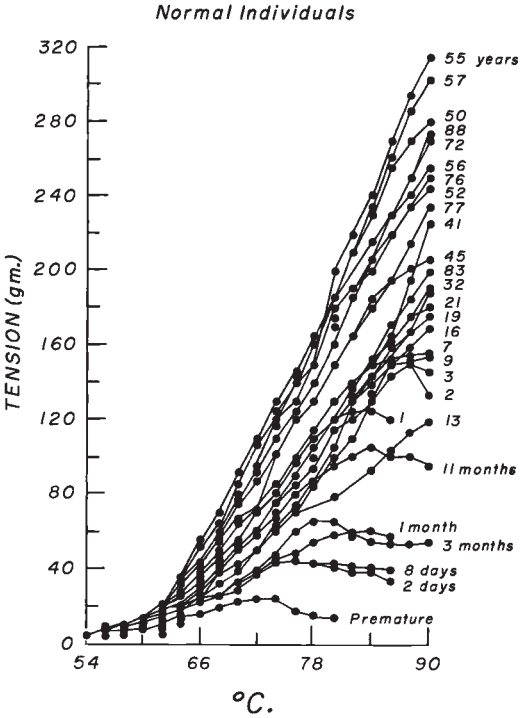

FIG. 4. Rate of tension development with temperature change in normal collagen.

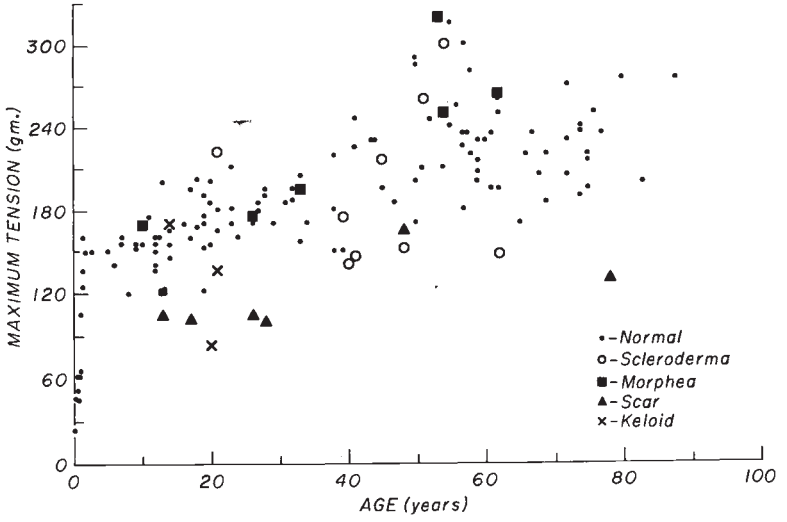

FIG. 5. Maximal tension developed by scleroderma, morphea, scars, and keloids compared to maximal tension of normal collagen.

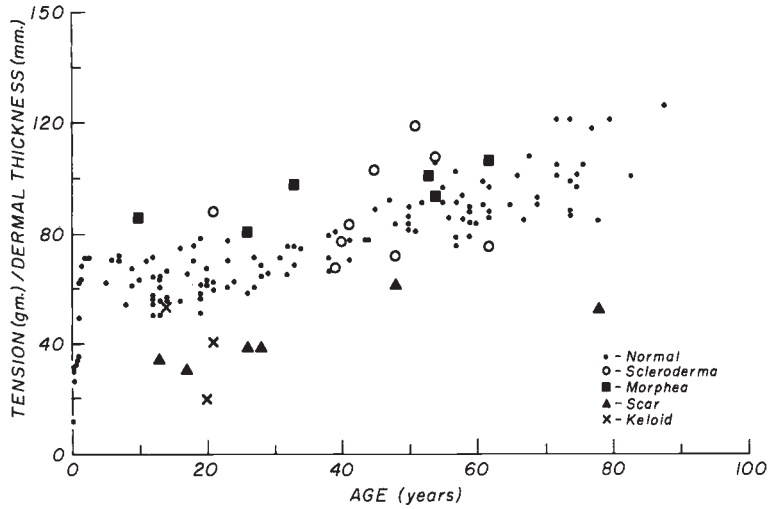

FIG. 6. Tension per unit dermal thickness developed by scleroderma, morphea, scars, and keloids compared to tension per unit dermal thickness of normal collagen.

normal aging curve some difference would be expected to exist for the life of the individual. This is consistent with the findings of Verzár and Willenegger (11) who found, by measuring the amount of extractable hydroxyproline, that scar tissue had the characteristics of young tissue, even when in an older individual. They also found that the "young" collagen aged according to the age of the scar with a difference existing for as long as 6 years between collagen in the scar and that in normal skin.

Sclerodermatous collagen, on the other hand, behaves like the collagen from normal individuals of a similar age. No significant difference was found between samples of localized scleroderma and those of acrosclerosis, and the tissue behaved the same regardless of the duration of sclerosis or the treatment employed. This is consistent with the findings of most investigators who have studied the two types of scleroderma (12, 13) and supports the findings of Ziff and co-workers (14) who found the urinary excretion of hydroxyproline in both localized and disseminated scleroderma to be the same as that in normal adult individuals. Hydroxyproline levels in infants, on the other hand, were found to be three to four times higher than either those in normal adults or in individuals with scleroderma.

The relatively higher tension values found for most of the sclerodermatous samples when compared to those for normal tissue most likely reflect the increased number of collagen fibers per unit of tissue. The small amount of contraction and the flat contraction curve found in the more sclerotic samples probably were the result of the sclerosis preventing maximal stretching of the fibers prior to heating and had nothing to do with the age of the collagen.

While collagen-turnover studies and precise chemical analysis will be necessary to establish the exact metabolic activity and chemical composition of sclerodermatous tissue, one can predict from the results presented here and from the data accumulating in the literature that the collagen from both localized and generalized scleroderma will not be unlike that of normal mature collagen. The final answer concerning the pathogenesis of scleroderma will probably come from approaches other than those involving direct study of the collagen itself.

SUMMARY

The shrinkage temperature, amount of contraction, and rate and amount of tension developed during isotonic and isometric hydrothermal contraction of dermal collagen were determined for samples of skin taken from 9 patients with scars or keloids and from 15 patients with either generalized or localized scleroderma.

Scars and keloids behaved like young collagen with a correlation between tension and the duration of the lesion. Sclerodermatous tissue

behaved like mature collagen with the tension generally being the same or slightly higher than that of normal collagen of a comparable age.

The findings suggest that collagen from scars and keloids represents new relatively unstable collagen and that collagen from sclerodermatous tissue is primarily mature stable collagen and not an overabundance of newly synthesized fibers as has been suggested by some investigators.

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